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(54) Title: METHOD OF PREVENTING OR TREATING DISEASE CHARACTERIZED BY NEOPLASTIC CELLS EXPRESSING CD40

(57) Abstract

There is disclosed a method of treating a mammal afflicted with a disease characterized by neoplastic cells that express CD40, comprising administering a therapeutically effective amount of a CD40 binding protein in a pharmaceutically acceptable buffer. CD40 binding proteins include monoclonal antibodies to CD40, and CD40 ligand. CD40 binding proteins may also be used to prevent disease characterized by neoplastic cells that express CD40, in individuals at risk for such disease.

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TITLE

METHOD OF PREVENTING OR TREATING DISEASE CHARACTERIZED BY NEOPLASTIC CELLS EXPRESSING CD40

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods of preventing or treating diseases characterized by neoplastic cells expressing CD40. More specifically, the present invention relates to methods of treating or preventing B-cell lymphomas.

BACKGROUND OF THE INVENTION

Immunoblastic B-cell lymphomas frequently arise in immunocompromised individuals such as allograft recipients and others receiving long-term immunosuppressive therapy, AIDS patients and patients with primary immunodeficiency syndromes such as X-linked lymphoproliferative syndrome or Wiscott-Aldrich syndrome (Thomas et al., *Adv. Cancer Res.* 57:329, 1991; Straus et al., *Ann. Intern. Med.* 118:45, 1993). These tumors appear to arise as a result of impaired T cell control of latent Epstein-Barr virus (EBV) infection. Similar lymphomas of human origin can be induced in mice with severe combined immunodeficiency syndrome (SCID) by inoculation of peripheral blood lymphocytes (PBL) from healthy, EBV-positive individuals (Mosier et al., *Nature* 335:256, 1988; Rowe et al., *J. Exp. Med.* 173:147, 1991).

CD40, a cell-surface antigen present on the surface of both normal and neoplastic human B cells, is a peptide of 277 amino acids having a predicted molecular weight of 30,600, with a 19 amino acid secretory signal peptide comprising predominantly hydrophobic amino acids. This cell surface antigen has been shown to play an important role in B-cell proliferation and differentiation. A cDNA encoding CD40 was isolated from a cDNA library prepared from Burkitt lymphoma cell line Raji (Stamenkovic et al., *EMBO J.* 8:1403, 1989). CD40 is also expressed on the surface of monocytic and epithelial cells, and on some epithelial carcinomas (E.A. Clark, *Tissue Antigens* 36:33; 1990).

Activated CD4+ T cells express high levels of a ligand for CD40 (CD40L). Human CD40L, a membrane-bound glycoprotein, has recently been cloned from peripheral blood T-cells as described in Spriggs et al., *J. Exp. Med.* 176:1543 (1992), and in United States Patent Application number 07/969,703, filed October 23, 1992, the disclosure of which is incorporated by reference herein. The cloning of murine CD40L is described in Armitage et al., *Nature* 357:80, 1992. CD40L induces B-cell proliferation in the absence of any co-stimulus, and can also induce production of immunoglobulins in the presence of cytokines.

Monoclonal antibodies to CD40 are known in the art (see, for example, the sections dedicated to B cell antigens in LEUKOCYTE TYPING III; A.J. McMichael ed. Oxford

University Press Oxford, and LEUKOCYTE TYPING IV: Oxford University Press. Oxford). Antibodies to CD40 have been demonstrated to exert costimulatory signals on normal B cells, resulting in proliferative and differentiation responses. Similarly, CD40-L exerts protein stimulatory or costimulatory signals to normal B cells.

It has been observed that cross-linking of surface IgM on some B cell lymphoma lines exerts inhibitory signals to the lymphoma cells (Beckwith et al., *J. Immunol.* 147:2411, 1991). Similarly, exposure of malignant B or T cells to stimuli that lead to activation of normal lymphocytes can result in growth arrest of the cells (Ashwell et al., *Science* 237:61, 1987; Bridges et al., *J. Immunol.* 139:4242, 1987; Mercep et al., *J. Immunol.* 140:324, 1988; Sussman et al., *J. Immunol.* 140:2520, 1988; Warner and Scott, *Cell. Immunol.* 115:195, 1988; Page and DeFranco, *J. Immunol.* 140:3717, 1988).

Garnier et al. observed that antibodies to CD40 or another B cell marker, CD23, showed some degree of effectiveness at inhibiting lymphoma formation in SCID mice that had been injected with human PBL and then infected with EBV (Abstract 167, XIVth Intl. Congress of the Transplantation Society, 1992). However, it was unknown in the art whether the mechanism of action involved was inhibition of binding of CD40L to CD40 by the anti-CD40 antibody, or by some other means. Therefore, there is a need in the art to determine the effects of other anti-CD40 antibodies, and of CD40-L itself, upon B cell lymphomas and other malignant cells that express CD40.

SUMMARY OF THE INVENTION

The present invention relates to a method of treating a mammal afflicted with a disease characterized by neoplastic cells that express CD40, comprising administering a therapeutically effective amount of a CD40 binding protein in a pharmaceutically acceptable buffer. The therapeutically effective amount is from about 0.01 to about 1 mg/kg body weight. CD40 binding proteins may be selected from the group consisting of monoclonal antibodies to CD40, CD40 ligand, and combinations thereof. Particularly preferred monoclonal antibodies are hCD40m2 (deposited at the American Type Culture Collection, Rockville, MD, USA, under the terms of the Budapest Treaty, and given ATCC accession number HB11459) and hCD40m3, which are described in U.S.S.N. 08/130, 541, filed October 1, 1993. Oligomeric forms of CD40 ligand are particularly preferred, and include a soluble CD40 ligand-Fc fusion protein, and an oligomeric CD40 leucine zipper fusion protein, both of which have been described in U.S.S.N. 07/969,703, filed October 23, 1992. The present invention also relates to a method of preventing a disease characterized by neoplastic cells that express CD40, in a mammal susceptible to the disease, comprising administering a therapeutically effective amount of a CD40 binding protein in a pharmaceutically acceptable buffer, wherein the therapeutically effective amount is from

about 0.01 to about 1 mg/kg body weight. Neoplastic cells that express CD40 include B lymphoma cells, some melanoma cells and some carcinoma cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the expression of CD40 by several lymphoma cell lines, using anti-CD40 monoclonal antibodies M2 and M3.

Figure 2 demonstrates the inhibition of the proliferation of several lymphoma cell lines by antibodies to CD40 (closed squares); in contrast, msIgG did not inhibit proliferation (open squares).

Figure 3 presents a comparison of the effects of soluble anti-CD40 (panel A) or immobilized anti-CD40 (panel B) on lymphoma growth.

Figure 4 demonstrates the ability of soluble CD40 ligand to inhibit the growth of B-cell lymphomas *in vitro*.

Figure 5 shows that antibodies to CD40 inhibit the growth of human melanoma cells *in vitro*.

Figure 6 demonstrates the ability of soluble CD40 ligand to inhibit the growth of B-cell lymphomas *in vivo*.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of treating or preventing diseases characterized by neoplastic cells that express a cell surface molecule known as CD40. The inventive methods utilize a protein (or proteins) that specifically bind CD40 (referred to as a CD40 binding protein) in a non-covalent interaction based upon the proper conformation of the CD40 binding protein and CD40 itself. For example, a CD40 binding protein can comprise an extracellular region of a CD40 ligand. In other cases, a CD40 binding protein can comprise an antibody that binds CD40 through an antigen binding region. Additional CD40 binding proteins can be prepared through recombinant methods, by preparation of fusion proteins comprising a CD40 binding region (or domain) from a CD40 ligand, or an antibody to CD40, with a second protein, for example, a human immunoglobulin Fc domain.

CD40

Human CD40 antigen (CD40) is a peptide of 277 amino acids having a molecular weight of 30,600, and a 19 amino acid secretory signal peptide comprising predominantly hydrophobic amino acids (Stamenkovic et al., *supra*). A cDNA encoding human CD40 was isolated from a cDNA library prepared from Burkitt lymphoma cell line Raji. The putative protein encoded by the CD40 cDNA contains a putative leader sequence, trans-membrane domain and a number of other features common to membrane-bound receptor

proteins. CD40 has been found to be expressed on B lymphocytes, epithelial cells and some carcinoma cell lines.

CD40 is a member of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor family, which is defined by the presence of cysteine-rich motifs in the extracellular region (Smith et al., *Science* 248:1019, 1990; Mallett and Barclay, *Immunology Today* 12:220; 1991). This family includes the lymphocyte antigen CD27, CD30 (an antigen found on Hodgkin's lymphoma and Reed-Sternberg cells), two receptors for TNF, a murine protein referred to as 4-1BB, rat OX40 antigen, NGF receptor, and *Fas* antigen.

CD40 may be detected on the surface of a cell by any one of several means known in the art. For example, an antibody specific for CD40 may be used in a fluorescence-activated cell sorting technique to determine whether cells express CD40, as described in Example 1 herein. Other methods of detecting cell surface molecules are also useful in detecting CD40.

CD40 Monoclonal Antibodies

Monoclonal antibodies directed against the CD40 surface antigen (CD40 mAb) have been shown to mediate various biological activities on human B cells. For example, CD40 mAb induce homotypic and heterotypic adhesions (Barrett et al., *J. Immunol. 146*:1722, 1991; Gordon et al., *J. Immunol. 140*:1425, 1988), and increase cell size (Gordon et al., *J. Immunol. 140*:1425, 1988; Valle et al., *Eur. J. Immunol. 19*:1463, 1989). CD40 mAb also induce proliferation of B cells activated with anti-IgM, CD20 mAb, or phorbol ester alone (Clark and Ledbetter, *Proc. Natl. Acad. Sci. USA 83*:4494, 1986; Gordon et al., LEUKOCYTE TYPING III; A.J. McMichael ed. Oxford University Press. Oxford, p. 426; Paulie et al., *J. Immunol. 142*:590, 1989) or in concert with IL-4 (Valle et al., *Eur. J. Immunol. 19*:1463, 1989; Gordon et al., *Eur. J. Immunol. 17*:1535, 1987), and produce IgE (Jabara et al., *J. Exp. Med. 172*:1861, 1990; Gascan et al., *J. Immunol. 147*:8, 1991), IgG, and IgM (Gascan et al., *J. Immunol. 147*:8, 1991) from IL-4-stimulated T cell-depleted cultures.

In addition, CD40 mAb have been reported to enhance IL-4-mediated soluble CD23/FcεRII release from B cells (Gordon and Guy, *Immunol. Today 8*:339, 1987; Cairns et al., *Eur. J. Immunol. 18*:349, 1988) and to promote B cell production of IL-6 (Clark and Shu, *J. Immunol. 145*:1400, 1990). Recently, in the presence of CD_w32+ adherent cells, human B cell lines have been generated from primary B cell populations with IL-4 and CD40 mAb (Banchereau et al., *Science 241*:70, 1991). Furthermore, germinal center centrocytes can be prevented from undergoing apoptosis if they are activated through CD40 and/or receptors for antigen (Liu et al., *Nature 342*:929, 1989). Each of the above publications describes CD40 mAb that stimulate a biological activity of B cells.

U.S.S.N. 08/130, 541, filed October 1, 1993, the relevant disclosure of which is incorporated by reference, discloses two monoclonal antibodies to CD40, referred to as hCD40m2 and hCD40m3. Unlike other CD40 mAb, hCD40m2 (ATCC HB11459) and hCD40m3 bind CD40 and inhibit binding of CD40 to cells that constitutively express CD40L. Greater than 95% inhibition of binding was observed with hCD40m2 or with CD40 mAb M3, at concentrations as low as 12.5μg/ml, as compared to irrelevant IgG or a control CD40 mAb, G28.5. hCD40m2 was also able to inhibit CD40L-induced TNF-α production.

Additional CD40 monoclonal antibodies may be generated using conventional techniques (*see* U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993 which are incorporated herein by reference; *see also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988, which are also incorporated herein by reference).

Briefly, an animal is injected with a form of CD40 suitable for generating an immune response against CD40. The animal may be reimmunized as needed until levels of serum antibody to CD40 have reached a plateau, then be given a final boost of soluble CD40, and three to four days later sacrificed. Organs which contain large numbers of B cells such as the spleen and lymph nodes are harvested and disrupted into a single cell suspension by passing the organs through a mesh screen or by rupturing the spleen or lymph node membranes which encapsulate the cells.

Alternatively, suitable cells for preparing monoclonal antibodies are obtained through the use of *in vitro* immunization techniques. Briefly, an animal is sacrificed and the spleen and lymph node cells are removed. A single cell suspension is prepared, and the cells are placed into a culture which contains a form of CD40, which is suitable for generating an immune response as described above. Subsequently, the lymphocytes are harvested and fused as described below.

Cells which are obtained through the use of *in vitro* immunization or from an immunized animal as described above may be immortalized by transfection with a virus such as the Epstein bar virus (EBV) (*see* Glasky and Reading, *Hybridoma 8(4)*:377-389, 1989). Alternatively, the harvested spleen and/or lymph node cell suspensions are fused with a suitable myeloma cell in order to create a "hybridoma" which secretes monoclonal antibody. Suitable myeloma lines are preferably defective in the construction or expression of antibodies, and are additionally syngeneic with the cells from the immunized animal. Many such myeloma cell lines are well known in the art and may be obtained from sources such as the American Type Culture Collection (ATCC), Rockville, Maryland (*see Catalogue of Cell Lines & Hybridomas*, 6th ed., ATCC, 1988).

CD40 Ligand

Activated CD4+ T cells express high levels of a ligand for CD40 (CD40L). Human CD40L, a membrane-bound glycoprotein, has recently been cloned from peripheral blood T-cells as described in Spriggs et al., *J. Exp. Med.* 176:1543 (1992), and in United States Patent Application number 07/969,703, filed October 23, 1992, the disclosure of which is incorporated by reference herein. The cloning of murine CD40L is described in Armitage et al., *Nature* 357:80, 1992. CD40L induces B-cell proliferation in the absence of any co-stimulus, and can also induce production of immunoglobulins in the presence of cytokines.

CD40-L is a type II membrane polypeptide having an extracellular region at its C-terminus, a transmembrane region and an intracellular region at its N-terminus. Soluble CD40-L comprises an extracellular region of CD40-L (amino acid 47 to amino acid 261 of SEQ ID NO:1) or a fragment thereof. CD40-L biological activity is mediated by binding of the extracellular region of CD40-L with CD40, and includes B cell proliferation and induction of antibody secretion (including IgE secretion).

U.S.S.N. 07/969,703 describes preparation of a soluble CD40-L/Fc fusion protein referred to as CD40-L/FC2. CD40-L/FC2 contains an eight amino acid hydrophilic sequence described by Hopp et al. (Hopp et al., *Bio/Technology* 6:1204,1988; referred to as Flag®), an IgG_1 Fc domain, a $[Gly_4Ser]_3$ linker sequence (described in U.S. Patent 5,073,627), and the extracellular region of human CD40-L. Also described in U.S.S.N. 07/969,703 is a soluble CD40-L fusion protein referred to as trimeric CD40-L., which contains a 33 amino acid sequence referred to as a "leucine zipper," the eight amino acid hydrophilic sequence described by Hopp et al. (*supra*), followed by the extracellular region of human CD40-L. Both oligomeric forms of CD40-L induce human B cell proliferation in the absence of any co-stimuli, and (in conjunction with the appropriate cytokine) result in the production of IgG, IgE, IgA and IgM.

The CD40-L/FC2 and the trimeric CD40-L described in U.S.S.N. 07/969,703 will be useful in the present inventive methods, as will other forms of CD40-L that can be prepared using known methods of preparing recombinant proteins.

Additional CD40 Binding Proteins

Binding proteins may also be constructed utilizing recombinant DNA techniques to incorporate the variable regions of a gene which encodes an antibody to CD40. (*see* James W. Larrick et al., "Polymerase Chain Reaction Using Mixed Primers: Cloning of Human Monoclonal Antibody Variable Region Genes From Single Hybridoma Cells," *Biotechnology* 7:934-938, September 1989; Reichmann et al., "Reshaping Human Antibodies for Therapy," *Nature 332*:323-327, 1988; Roberts et al., "Generation of an Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering," *Nature 328*:731-734, 1987; Verhoeyen et al., "Reshaping Human Antibodies: Grafting an

Antilysozyme Activity," *Science 239*:1534-1536, 1988; Chaudhary et al., "A Recombinant Immunotoxin Consisting of Two Antibody Variable Domains Fused to *Pseudomonas* Exotoxin," *Nature 339*:394-397, 1989).

Briefly, DNA encoding the antigen-binding site (or CD40 binding domain; variable region) of a CD40 mAb is isolated, amplified, and linked to DNA encoding another protein, for example a human IgG (*see* Verhoeyen et al., *supra*; *see also* Reichmann et al., *supra*). Alternatively, the antigen-binding site (variable region) may be either linked to, or inserted into, another completely different protein (*see* Chaudhary et al., *supra*), resulting in a new protein with antigen-binding sites of the antibody as well as the functional activity of the completely different protein.

Furthermore, DNA sequences which encode smaller portions of the antibody or variable regions which specifically bind to mammalian CD40 may also be utilized within the context of the present invention. Similarly, the CD40 binding region (extracellular domain) of a CD40 ligand may be used to prepare other CD40 binding proteins. DNA sequences that encode proteins or peptides that form oligomers will be particularly useful in preparation of CD40 binding proteins comprising an antigen binding domain of CD40 antibody, or an extracellular domain of a CD40 ligand. Certain of such oligomer-forming proteins are disclosed in U.S.S.N. 07/969,703; additional, useful oligomer-forming proteins are also disclosed in U.S.S.N. 08/107,353, filed August 13, 1993, and in U.S.S.N. 08/145,830, filed September 29, 1993.

Once suitable antibodies or binding proteins have been obtained, they may be isolated or purified by many techniques well known to those of ordinary skill in the art (*see Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988). Suitable techniques include peptide or protein affinity columns, HPLC or RP-HPLC, purification on protein A or protein G columns, or any combination of these techniques. Recombinant CD40 binding proteins can be prepared according to standard methods, and tested for binding specificity to the CD40 utilizing assays known in the art, including for example ELISA, ABC, or dot blot assays, as well by bioactivity assays such as those described for CD40 mAb.

SCID mouse models

The term SCID (severe combined immune deficiency) mouse refers to a mutant C.B-17 strain of mouse with a chromosome 16 deficiency that prevents correct T cell receptor and immunoglobulin gene rearrangement, and is thus virtually devoid of functional B and T cells (Bosma et al., *Nature* 301:257; 1983). SCID mice can be successfully reconstituted with human fetal lymphoid tissues, and with adult human lymphocytes, and have thus been useful as a model for studying human immune function *in vivo* (Mosier et al., *Nature* 335:256, 1988; McKune et al., *Science* 241:1632, 1988; Kamel-Reid and Dick,

Science 242:1707, 1988). SCID mice reconstituted with human peripheral blood lymphocytes (PBL) from individuals with serological evidence of Epstein-Barr virus (EBV) infection often develop lymphomas of B cell origin (Mosier et al. *supra*; Cannon et al., *J. Clin. Invest.* 85:1333, 1990; Rowe et al., *J. Exp. Med.* 173:147, 1991; Purtilo et al., *Int. J. Cancer* 47:510, 1991). Veronese et al. reported that the presence of functional T cells in the injected PBL was absolutely necessary for progression of latently EBV-infected B cells into tumor masses (*J. Exp. Med.* 176:1763, 1992). The lymphomas that develop in this SCID mouse model are highly aggressive, and analogous to EBV-lymphomas that arise in immunocompromised individuals.

Administration of CD40 Binding Protein Compositions

The present invention provides therapeutic compositions comprising an effective amount of a CD40 binding protein in a suitable diluent or carrier and methods of treating mammals using the compositions. For therapeutic use, purified CD40 binding protein or a biologically active analog thereof is administered to a patient, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, CD40 binding protein pharmaceutical compositions (for example, in the form of a soluble extracellular domain of CD40 ligand, or a fragment thereof, or a monoclonal antibody to CD40) which is administered to achieve a desired therapeutic effect can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique.

Typically, a CD40 binding protein therapeutic agent will be administered in the form of a pharmaceutical composition comprising purified CD40 binding protein in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to patients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining a CD40 binding protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents.

Appropriate dosages may be determined by methods that are known in the art. Typically, therapeutically effective dosages of CD40 binding proteins will be in the range of from about 0.01 to about 1 mg/kg body weight. Moreover, CD40 binding proteins may also be used in conjugates of, or combination with, drugs, toxins or radioactive compounds. Preparation of such conjugates for treatment of various diseases are known in the art (see, for example, Waldmann, *Science* 252:1657, 1991).

Prevention or treatment

These results presented herein indicate that CD40 binding proteins may be of significant clinical use not only in the treatment of B-cell lymphomas, but also in the prevention of EBV-induced B-cell lymphoma that can occur after transplantation or in other instances of immunosuppression, such as AIDS, and which present a significant risk in such patient populations. Since CD40 binding proteins can inhibit various B-cell lymphomas directly, it may not be necessary to use them in conjugates of toxins or radioactive compounds, thereby avoiding toxicity and potential negative effects on normal B cells.

The inventive methods may be useful in prevention of immunoblastic B-cell lymphomas that frequently arise in immunocompromised individuals. In such preventative methods, a mammal at risk of developing an immunoblastic B-cell lymphoma is administered CD40 binding protein. The CD40 binding proteins can be administered for as long as the state of immunocompromise that places the individual at risk exists.

Similarly, the results indicate that the inventive methods may be used to prevent occurrence (or reoccurrence) of neoplastic disease characterized by other types of malignant cells that express CD40 in individuals at risk for such disease. Individuals that are considered at risk in these instances include those with family history or other genetic characteristics indicating predisposition to cancers in which the neoplastic cells express CD40, and individuals that develop drug-resistant neoplastic disease as a result of chemotherapy, in which the drug-resistant neoplastic cells express CD40.

Individuals afflicted with disease characterized by neoplastic cells that express CD40 may also be treated according to the inventive methods. The term treatment, as it is generally understood in the art, refers to initiation of therapy after clinical symptoms or signs of disease have been observed. The inventive methods may be used in conjunction with other therapies appropriate for afflicted individuals, including chemotherapy, radiation therapy, and immunotherapy.

The relevant disclosures of all references cited herein are specifically incorporated by reference. The following examples are intended to illustrate particular embodiments, and not limit the scope, of the invention.

Example 1

This example describes the characterization of human B-cell lymphoma cells and cell lines. Cells used included RL and DB, cell lines obtained from patients with diffuse, large cell lymphomas of B-cell origin (Beckwith et al., supra), and TU2C and CHIM62, EBV-induced lymphomas obtained from SCID mice that had been injected with PBL from EBV-seropositive individuals. These cells were maintained in culture under standard

culture conditions for less than six months prior to initiation of the study. Other cells included Raji, a cell line cultured from a patient with Burkitt's lymphoma, and LCL-2311, a lymphoblastoid cell line generated by infecting human PBL with EBV *in vitro* .

All of the cell lines were positive for CD40 expression by flow cytometry, using anti-CD40 monoclonal antibodies M2 and M3; results are shown in Figure 1. RL, DB, and Raji cells were homogeneous in their expression of CD40, whereas the EBV-induced lymphomas from SCID mice were heterogeneous in the staining intensity with anti-CD40. Lymphomas from these mice have previously been demonstrated to be heterogeneous and oligoclonal (Nakamine et al., *Am J. Pathol.* 142:139, 1993), which may account for the differential expression of CD40. CD20, another B cell marker, was also present on the tumor cells.

Example 2

This example describes the effect of anti-CD40 antibodies (M2 and M3) on the proliferative potential of lymphoma cells and cell lines *in vitro*. Proliferation was determined using an assay substantially as described by Rowe et al. (*J. Exp. Med.* 173:147, 1991). Briefly, cell lines were split 24 hours before assays were performed. Cells were resuspended in culture medium to a concentration of 1 x 10^5/ml, and 100 µl of cell suspension was plated in 96-well, round bottom microtiter plates (Corning Glass Works, Corning NY, USA) already containing 100 µl of appropriately diluted reagents (monoclonal anti-CD40 antibodies M2 and M3, obtained from Immunex Corporation, Seattle, WA, USA, or mouse IgG myeloma protein (msIgG) purchased from Cappel, Westchester, PA, USA). Seventy-two hours later, 1 µCi of [^{3}H]-thymidine/well (specific activity 6.7 Ci/mmol; New England Nuclear Research Products, Boston, MA, USA) was added for the final 8 to 18 hours of culture. Cultures were harvested onto glass fiber filters with a PhDCell Harvesting System (Cambridge Technology Inc., Cambridge, MA USA), and [^{3}H]-thymidine uptake was assayed by liquid scintillation using an LKB ß-counter (LKB Instruments Inc., Turku, Finland). Each experiment was performed four to six times, with the results of a representative experiment being presented in Figure 2.

Incubation with anti-CD40 monoclonal antibodies M2 and M3 resulted in significant inhibition of the proliferation of RL, DB, LCL-2311 and EBV-lymphoma cell lines tested, with an optimal inhibition of 40-60% occurring at 1-10 µg/ml of soluble antibody, depending on the lymphoma cell. The Raji cell line did not appear to be significantly affected by soluble anti-CD40.

The effects of soluble anti-CD40 on lymphoma growth were then compared to those of immobilized anti-CD40. Briefly, wells were incubated overnight at 37°C with goat anti-mouse antibody. Monoclonal anti-CD40 antibodies M2, M3, an anti-CD20 monoclonal antibody provided by Dr. Kevin Conlon (Laboratory of Experimental

Immunology, BRMP, NCI-FCRDC, Frederick, MD, USA), or msIgG, at a concentration of 10 μg/ml, were then added to he wells, and the wells were incubated for an additional 4 hours at 37°C. The proliferation assays were then performed as described above; results are shown in Figure 3. Immobilization resulted in significantly greater inhibition of proliferation ($p<0.05$) by the immobilized anti-CD40 antibodies as compared to soluble anti-CD40, or soluble or immobilized anti-CD20. Thus, in contrast to it's effects on normal B cells, stimulation of CD40 exerts inhibitory effects on EBV-induced B lymphomas.

Example 3

This example illustrates the effect of CD40 ligand on the growth of B-cell lymphomas *in vitro*. Soluble CD40 ligand (CD40-L; described in U.S.S.N. 07/969,703) was obtained from transfected COS-7 cells as supernatant fluid, and tested in a proliferation assay used as described above, in Example 2, using RL or TU2C cells. Both murine and human CD40-L-containing supernatant fluids were tested, since murine CD40-L binds to human cells that express CD40, and acts as a costimulus in the same manner as human CD40-L. Each lot of supernatant fluid was titrated to determine the concentration that yielded optimal inhibition of proliferation; a 1:5 dilution yielded maximal inhibition.

Exemplary results are presented in Figure 4; values are presented as percent of inhibition compared to control supernatant fluids. The soluble human ligand was inhibitory for the various lymphomas tested, with maximal inhibition seen (50-80%) on RL and TU2C cell lines at a 1:5 dilution of the supernatant fluid. The soluble murine CD40-L produced similar, if not better, inhibitory effects. Control supernatant fluid from COS-7 cells transfected with vector alone actually promoted lymphoma cell growth. Accordingly, the inhibitory effects of CD40-L on B lymphomas parallels that of antibodies to CD40.

Example 4

This example illustrates the effect of anti-CD40 on the growth of human B-cell lymphomas in SCID mice. C.B-17 *scid/scid* (SCID) mice were obtained from the Animal Production Facility (NCI-FCRDC, Frederick, MD, USA) and were not used until 6-8 weeks of age. The mice were kept under specific-pathogen-free conditions at all times; they housed in microisolator cages, and all food, water and bedding were autoclaved before use. Trimethoprim/sulfamethoxazole (40 mg trimethoprim and 200 mg sulfamethoxazole per 320 ml) was included in suspension form in the drinking water given to the mice. All mice received antisera to asialo GM1 (Wako Chemical, Dallas, TX, USA), a marker present on murine NK cells (Murphy et al., *Eur. J. Immunol.* 22:1421; 1992) intravenously one day before cell transfer, to remove host resistance to the tumor.

On day 0, SCID mice were injected either intravenously or intraperitoneally with 5 x 10^6 RL or TU2C cells. The tumor cell recipients then received either 2 μg of anti-CD40, or msIgG in 0.2 ml HBSS (Hank's balanced salt solution) intravenously every other day for a period of 10 days (total of 5 injections), starting at day 0, 3, or 14. Mice were monitored for tumor development and progression; moribund mice were euthanized. All mice were necropsied for evidence of tumor. Liver, kidney and lymphoid organs were analyzed histologically for presence of tumor cells. Both parametric (student's t test) and non-parametric (Wilcoxan rank sum test) analyses were performed to determine if the groups differed significantly ($p<0.05$). All experiments had 3-10 mice per group, and were performed 2-3 times. The results are presented in Table 1 below.

Table 1: Effect of anti-CD40 administration on survival in tumor-bearing mice

Experiment	Tumor (Route)	Treatment (Initiation)	No. of Mice	Mean Day of Death
1	RL (i.p.)	None	3	34±0
	RL (i.p.)	anti-CD40 (day 0)	6	>138±26.5[a]
	TU2C (i.p.)	None	3	28±0
	TU2C (i.p.)	anti-CD40 (day 0)	6	>76±45.0[b]
2	TU2C (i.v.)	None	6	30±6.3
	TU2C (i.v.)	anti-CD40 (day 3)	6	>38±2.6[c]
	TU2C (i.v.)	anti-CD40 (day 14)	6	>32±4.6[c]
3	RL (i.p.)	None	6	39±3.5
	RL (i.p.)	anti-CD40 (day 3)	6	>107±21.9[a]
	RL (i.p.)	anti-CD40 (day 14)	6	>79±34.3[d]

a: No deaths due to tumor; all (6) mice surviving
b: Two deaths due to tumor, other (4 out of 6 mice) showing no evidence of tumor.
c: One of six recipient mice showing no evidence of disease.
d: Three deaths due to tumor; three mice surviving.

Anti-CD40 significantly ($p<0.05$) improved survival of mice receiving either RL or TU2C tumors when treatment was initiated on day 0, 3 or 14. When SCID mice were treated with anti-CD40 on day 0, no evidence of tumor was present in the mice receiving the RL B-cell lymphoma line after several months. However, some mice receiving the EBV lymphoma TU2C and anti-CD40 did develop tumor several weeks after cessation of anti-CD40 treatment.

Differential patterns of metastatic growth were observed for the different routes of tumor cell administration. Mice receiving the EBV-induced lymphomas i.p. developed peritoneal tumors with extensive metastases in the lymph nodes and liver, whereas mice receiving the lymphomas i.v. primarily developed renal metastases. Anti-CD40 was capable of significantly inhibiting tumor grown and promoting survival of recipient mice regardless of the route of tumor administration.

Treatment of tumor-bearing mice with anti-CD40 also resulted in significantly improved survival when treatment was initiated 3 or 4 days after tumor cell transfer, and even as late as 14 days. These results indicate that anti-CD40 treatment was also efficacious when treatment was initiated with relatively large and extensive tumor burdens (>1 cm^3) in the recipient mice.

Example 5

This example illustrates the effect of anti-CD40 on the growth of tumors in SCID mice injected with PBL from EBV-seropositive individuals. SCID mice (described in Example 4 above) were given injections of recombinant human growth hormone (rhGH: Genentech, South San Franciso, CA, USA), which has been demonstrated to promote EBV-lymphogensis in huPBL-SCID mice (Murphy et al., *Brain Behav. Immun.* 6:355; 1992), presumable due to promotion of human T-cell engraftment in treated mice (Murphy et al., *Proc. Natl. Acad. Sci. USA* 89:4481; 1992). T-cell engraftment appears to be essential for human B-cell lymphoma formation in the huPBL-SCID model (18). rhGH (10 μg in 0.2 ml HBSS) was given i.p. on day 0 and every other day until time of assay 4-8 weeks later. Human PBLs were obtained from healthy donors in leukopacks. Anti-asialo GM-1 was administered as described for Example 4.

Human PBL were obtained from healthy, EBV-seropositive donors in leukopacks. All donors were screened for antibodies to human immunodeficiency virus type 1 (HIV-1) and for hepatitis B surface antigen (HBsAg), and provided informed consent before donation. The PBL were purified by counter-current elutriation, and the lymphocyte fraction, containing >90% lymphocytes as assessed by flow cytometry, were obtained. The PBL (1×10^8) were injected i.p. into recipient SCID mice on day 0.

Mice were treated with anti-CD40, anti-CD20, or with msIgG (2 μg/0.2 ml PBS) i.p. every other day for 20 days for a total of 10 injections. Table 2 presents results representative of three experiments with 5-8 mice per group.

Table 2: Effect of anti-CD40 administration of EBV-induced B-cell lymphoma development in huPBL-SCID mice chimeras

Experiment	Treatment	No. of Mice	Mean Day of Death	% Incidence of Lymphoma[a]
A	msIgG	6	31.4±3.9	100%
	anti-CD40	6	>50[b]	0%
B	msIgG	8	26.3±2.1[c]	87%
	anti-CD40	8	>55[b]	0%
C	msIgG	5	33.2±1.2	100%
	anti-CD40	5	>53[b]	0%
	anti-CD20	5	>53	0%

a: Mice were moribund with evidence of extensive tumor nodules in the peritoneal cavity and evidence of lymphoma by histological assessment.

b: Treatment with anti-CD40 resulted in no deaths due to lymphoma and no evidence of lymphoma when assayed 2-6 weeks after cessation of anti-CD40 treatment. Anti-CD40 or anti-CD20 significantly ($p<0.001$) increased survival compared to control recipients.

c: One out of 8 mice surviving and showing no evidence of tumor.

The results demonstrated that treatment of huPBL-SCID chimeric mice with anti-CD40 at the time of huPBL transfer completely prevented the development of human B-cell lymphomas in the mice. Although anti-CD20 had no effect on the lymphomas *in vitro*, treatment of the huPBL-SCID chimeras with anti-CD20 also prevented the occurrence of lymphoma.

Example 6

This example illustrates the effect of anti-CD40 on the engraftment of human T (HLA^+, $CD3^+$) and B (HLA^+, $CD3^-$) cells in SCID mice. huPBL-SCID mice chimeras were prepared as described in Example 5 above. Percentages of human T and B cells found in the peritoneal cavity were determined, and the human immunoglobulin in the serum quantitated by enzyme-linked immunosorbent assay (ELISA). Animals were also examined for the presence of lymphoma; those animal that evidenced lymphoma were moribund, with evidence of extensive tumor nodules in the peritoneal cavity. Mice were assayed 4-8 weeks after huPBL transfer, with all control (msIg) treated mice succumbing to EBV-induced B cell lymphoma at day 33.2±1.2.

Single cell suspensions of peritoneal cavity cells were obtained, and evaluated by flow cytometry (FACS). Staining was performed in the presence of 2% human AB serum (Gibco BRL, Grand Island, NY, USA) to saturate human and mouse Fc receptors. Reagents used in the FACS analysis were monoclonal anti human-HLA-ABC conjugated to fluorescein isothiocyanate (FITC; Olympus, Lake Success, NY, USA), and Leu4-biotinylated anti-CD3 (Becton-Dickinson, Mountain View, CA, USA). After primary antibody incubation, cells were analyzed using an EPICS flow cytometer.

Human immunoglobulin levels were assayed by ELISA. Flat-bottom 96-well microtiter plates (Corning Glass Works, Corning, NY, USA) were coated with goat anti-human Ig (Kirkegaard and Perry Laboratory, Gaithersburg, MD, USA) at 1 μg/ml in PBS, washed twice and blocked with 5% goat serum. The wells were then incubated with sera obtained from the huPBL-SCID mice chimeras, or a titration of human IgM+IgG standard (DAKO Corp., Santa Barbara, CA, USA). After washing four times, alkaline phosphatase-conjugated goat anti-human Ig (Kirkegaard and Perry Laboratory, Gaithersburg, MD, USA) was added. The plates were incubated, and washed again. After the final wash, substrate was added, and the enzyme reaction allowed to develop. OD was measured at 402 nm. Results are presented in Table 3.

Table 3: Effect of anti-CD40 on human B-cell engraftment and EBV-induced development in huPBL-SCID chimera mice

Experiment	Animal No.	Treatment	# Peritoneal Cells (x 10^6)	% cells HLA^+, $CD3^-$	% cells HLA^+, $CD3^+$	Human serum Ig (μg/ml)	Presence of Lymphoma
A	1	msIgG	1.6	1.8	10.3	90	+
	2	msIgG	1.4	0.8	2.8	120	+
	3	msIgG	1.1	0.6	0.4	155	+
	4	msIgG	1.5	0.6	1.4	406	+
	5	msIgG	0.6	2.3	10.7	500	+
	6	anti-CD40	0.8	2.8	6.1	2.7	-
	7	anti-CD40	1.4	1.0	1.1	0.8	-
	8	anti-CD40	1.8	2.7	5.2	2.7	-
	9	anti-CD40	1.4	6.1	10.5	5.0	-
	10	anti-CD40	1.2	2.0	5.2	0.2	-
B	1	anti-CD40	9.0	6.1	7.2	11.7	-
	2	anti-CD40	2.1	0.3	0.9	1.0	-
	3	anti-CD40	1.8	0.5	6.7	15.0	-
	4	anti-CD40	0.6	5.1	5.0	4.0	-
	5	anti-CD40	2.0	0.9	2.4	5.0	-
C	1	anti-CD20	0.8	0.0	8.1	6.5	-
	2	anti-CD20	1.4	0.0	1.1	10.3	-
	3	anti-CD20	1.8	0.0	5.2	4.8	-
	4	anti-CD20	1.4	0.0	0.8	7.9	-
	5	anti-CD40	1.2	10.3	0.0	412.0	-
	6	anti-CD40	9.0	1.5	0.0	86.4	-
	7	anti-CD40	2.1	5.1	22.9	1110.0	-
	8	anti-CD40	1.8	0.6	0.9	18.5	-

Treatment with anti-CD40 did not prevent the engraftment of human T and B cells, as determined by FACS analysis of peritoneal cells, and determination of serum levels of human immunoglobulin. In Experiment A, the extent of human cell engraftment appeared quantitatively less in anti-CD40 treated animals than in animals receiving msIgG, based on the levels of human Ig in the serum. However, since the huPBL-SCID chimeric mice that did not receive anti-CD40 developed B-cell lymphomas, the high levels of human immunoglobulin detected are likely to be due to the B-cell lymphoma. In contrast to anti-CD40, treatment with anti-CD20 appeared to inhibit engraftment of B cells, both as indicated in lower percentages of B cells and in decreased levels of human Ig in the serum.

Example 7

This example illustrates the effect of anti-CD40 and msIgG on the engraftment of B cells in SCID mice. huPBL-SCID mice chimeras were prepared as described in Example 5 above, except that the PBLs were obtained from EBV-negative donors. The chimeras were thus not expected to develop lymphomas, thereby providing a more accurate indication of engraftment of normal B cells. The chimeras were treated with either anti-CD40 or anti-CD20, and percentages of human T and B cells found in the peritoneal cavity were determined, and the amount of human immunoglobulin in the serum quantitated as described in Example 6. Results are shown in Table 5.

Table 5: Effect of Anti-CD40 Treatment on Human Immunoglobulin Production in huPBL-SCID Chimeras

Animal #	Treatment[a]	Serum Immunoglobulin (μg/ml)
1.	huPBL, msIgG	125.0
2.	huPBL, msIgG	150.0
3.	huPBL, msIgG	1.7
4.	huPBL, msIgG	94.0
5.	huPBL, msIgG	112.0
6.	huPBL, msIgG	18.8
7.	huPBL, anti-CD40	194.0
8.	huPBL, anti-CD40	269.0
9.	huPBL, anti-CD40	239.0
10.	huPBL, anti-CD40	119.0
11.	huPBL, anti-CD40	206.0
12.	huPBL, anti-CD40	150.0

a: SCID mice received 100 million huPBL i.p. with 2 μg of anti-CD40 or msIgG given i.p. every other day for 20 days. Mice were analyzed for serum immunoglobulin levels using a human immunoglobulin-specific ELISA 3-4 weeks after huPBL transfer.

Treatment with anti-CD40 did promote engraftment of human B cells, indicating that anti-CD40 has additional value for treatment or prevention of lymphomas due to its ability to remove lymphoma cells while sparing normal B cells.

Example 8

This example illustrates the effect of antibodies to CD40 on the growth of human melanoma cells *in vitro*. Antibody to CD40 ligand (M2) was tested in a proliferation assay substantially as described above, in Example 2, using M16 human melanoma cells, which express CD40.

The results obtained are presented in Figure 5; values are presented as percent of inhibition compared to control supernatant fluids. Incubation with anti-CD40 monoclonal antibody M2 resulted in significant inhibition of the proliferation of M16 human melanoma cell line tested, with as little as 0.1 ng/ml causing inhibition of almost 50%. Increased inhibition was observed with increasing concentration of anti-CD40.

Example 9

This example illustrates the effect of recombinant human CD40 ligand on the growth of human B-cell lymphomas in SCID mice. SCID mice were obtained, and treated substantially as described in Example 4, above. On day 0, SCID mice were injected either intraperitoneally with 5 x 10^6 RL or TU2C cells. The tumor cell recipients then received 100 µl of concentrated supernatant fluid from cells transfected with either a vector encoding human CD40 ligand, or vector alone (control). Two concentrations of the CD40 ligand-containing supernatant fluid were tested: a ten-fold concentrate and a two-fold concentrate (10x and 2x, respectively). The concentrated supernatants were administered intraperitoneally every third day for a period of 15 days (total of 5 injections), starting at day 3. Mice were monitored for tumor development and progression; moribund mice were euthanized. All mice were necropsied for evidence of tumor. Liver, kidney and lymphoid organs were analyzed histologically for presence of tumor cells. Both parametric (student's t test) and non-parametric (Wilcoxan rank sum test) analyses were performed to determine if the groups differed significantly ($p<0.05$). All experiments had 7-10 mice per group, and were performed 3 times.

The results of an exemplary experiment utilizing RL cells are shown in Figure 6. Similar to the results obtained *in vitro* in Example 3, recombinant CD40 ligand inhibited the growth of tumor cells *in vivo* in SCID mice.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ARMITAGE, RICHARD
FANSLOW, WILLIAM
LONGO, DAN L.
MURPHY, WILLIAM

(ii) TITLE OF INVENTION: METHOD OF PREVENTING OR TREATING DISEASE CHARACTERIZED BY NEOPLASTIC CELLS EXPRESSING CD40

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 51 UNIVERSITY STREET
(C) CITY: SEATTLE
(D) STATE: WASHINGTON
(E) COUNTRY: USA
(F) ZIP: 98101

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Macintosh
(C) OPERATING SYSTEM: Apple Macintosh System 7.1
(D) SOFTWARE: Microsoft Word for Macintosh, Version #5.1a

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: December 21, 1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: USSN 08/172,664
(B) FILING DATE: December 23, 1993
(C) CLASSIFICATION:

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(C) REFERENCE/DOCKET NUMBER: 2818

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(A) TELEPHONE: (206)587-0430
(B) TELEFAX: (206)233-0644

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 840 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: CD40-L

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..831

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 261 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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CLAIMS

What is claimed is:

1. A method of treating a mammal afflicted with a disease characterized by neoplastic cells that express CD40, comprising administering a therapeutically effective amount of a CD40 binding protein in a pharmaceutically acceptable buffer, wherein the therapeutically effective amount is from about 0.01 to about 1 mg/kg body weight.

2. The method of claim 1, wherein the CD40 binding protein is selected from the group consisting of monoclonal antibodies to CD40, CD40 ligand, and combinations thereof.

3. The method of claim 2, wherein the CD40 binding protein is monoclonal antibody hCD40m2 (ATCC HB11459).

4. The method of claim 2, wherein the CD40-binding protein is monoclonal antibody hCD40m3.

5. The method of claim 2, wherein the CD40-binding protein is soluble, oligomeric CD40 ligand.

6. The method of claim 5, wherein the soluble, oligomeric CD40 ligand is an Fc fusion protein.

7. The method of claim 5, wherein the soluble, oligomeric CD40 ligand is a leucine zipper fusion protein.

8. The method of claim 1, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

9. The method of claim 2, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

10. The method of claim 3, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

11. The method of claim 4, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

12. The method of claim 5, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

13. The method of claim 6, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

14. The method of claim 7, wherein the cells expressing CD40 are selected from the group consisting of B lymphoma cells, melanoma cells and carcinoma cells.

15. A method of preventing a disease characterized by neoplastic cells that express CD40, in a mammal susceptible to the disease, comprising administering a therapeutically effective amount of a CD40 binding protein in a pharmaceutically acceptable buffer, wherein the therapeutically effective amount is from about 0.01 to about 1 mg/kg body weight.

16. The method of claim 15, wherein the CD40 binding protein is selected from the group consisting of monoclonal antibodies to CD40, CD40 ligand, and combinations thereof.

17. The method of claim 16, wherein the CD40 binding protein is monoclonal antibody hCD40m2 (ATCC HB11459).

18. The method of claim 16, wherein the CD40-binding protein is monoclonal antibody hCD40m3.

19. The method of claim 16, wherein the CD40-binding protein is soluble, oligomeric CD40 ligand.

20. The method of claim 19, wherein the soluble, oligomeric CD40 ligand is an Fc fusion protein.

21. The method of claim 19, wherein the soluble, oligomeric CD40 ligand is a leucine zipper fusion protein.

22. The use of a CD40 binding protein in providing a medication for administration to a mammal afflicted with a disease characterized by neoplastic cells that express CD40.

23. The use of claim 22, wherein the CD40 binding protein is selected from the group consisting of monoclonal antibodies to CD40, CD40 ligand, and combinations thereof.

24. The use of claim 23, wherein the CD40 binding protein is monoclonal antibody hCD40m2 (ATCC HB11459).

25. The use of claim 23, wherein the CD40-binding protein is monoclonal antibody hCD40m3.

26. The use of claim 23, wherein the CD40-binding protein is soluble, oligomeric CD40 ligand.

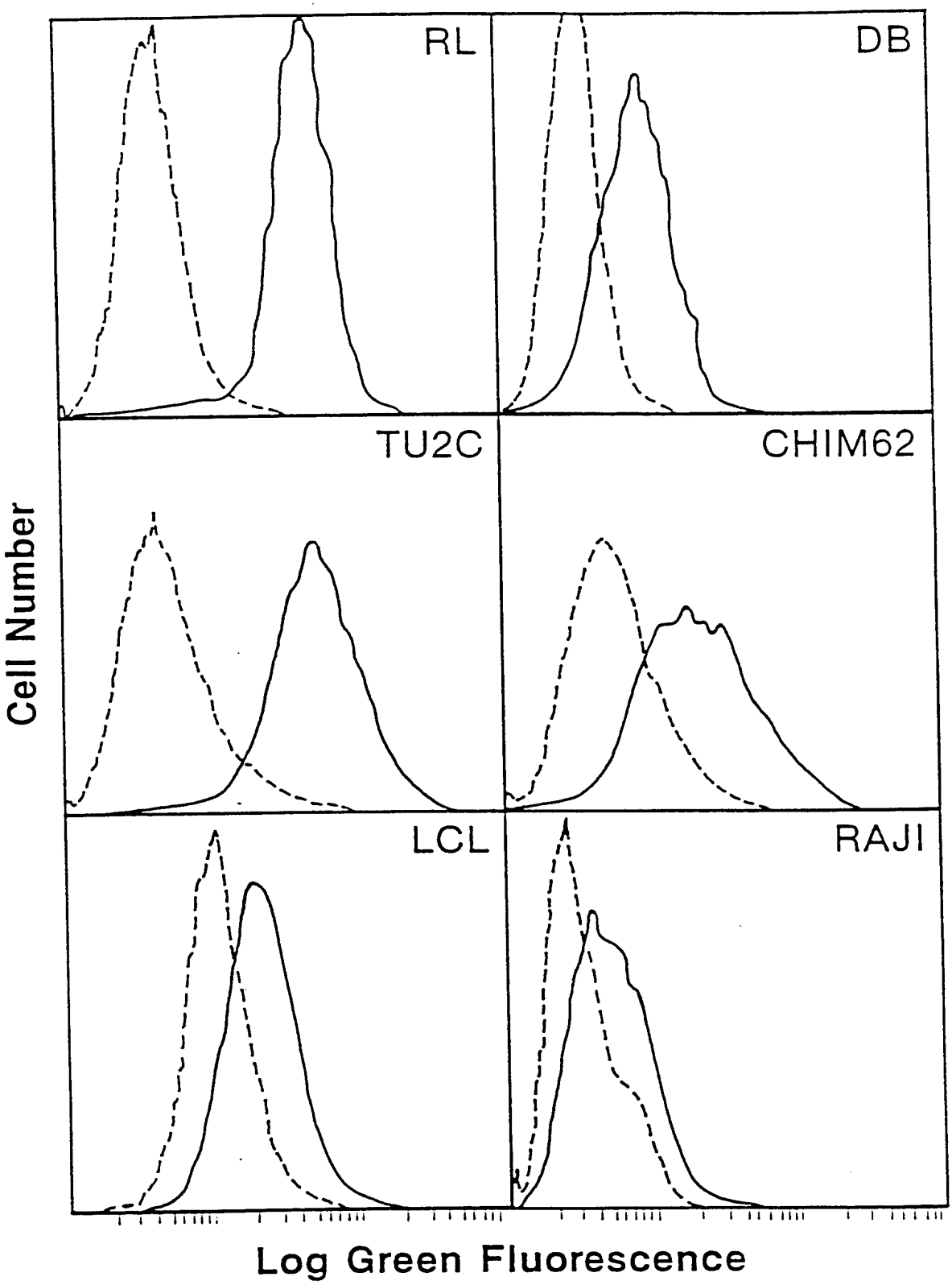
27. A method of preparing a medication for administration to a mammal afflicted with a disease characterized by neoplastic cells that express CD40, comprising formulating a CD40 binding protein in a suitable excipient or carrier.

28. The method of claim 27, wherein the CD40 binding protein is selected from the group consisting of monoclonal antibodies to CD40, CD40 ligand, and combinations thereof.

29. The method of claim 28, wherein the CD40 binding protein is monoclonal antibody hCD40m2 (ATCC HB11459).

30. The method of claim 28, wherein the CD40-binding protein is monoclonal antibody hCD40m3.

31. The method of claim 28, wherein the CD40-binding protein is soluble, oligomeric CD40 ligand.


RL
DB
TU2C
CHIM62
LCL
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Cell Number
Log Green Fluorescence

FIGURE 1

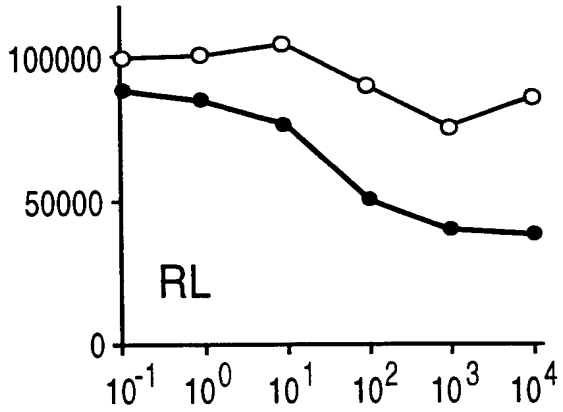

Figure 2A

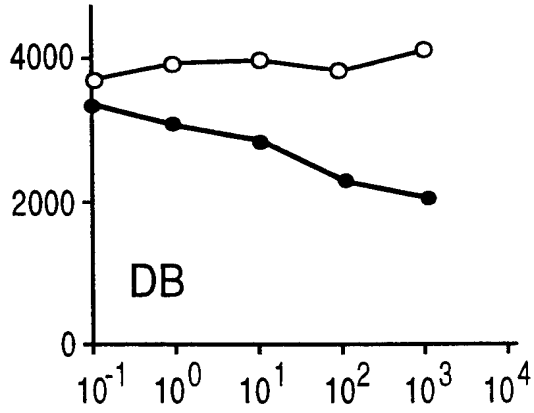

Figure 2B

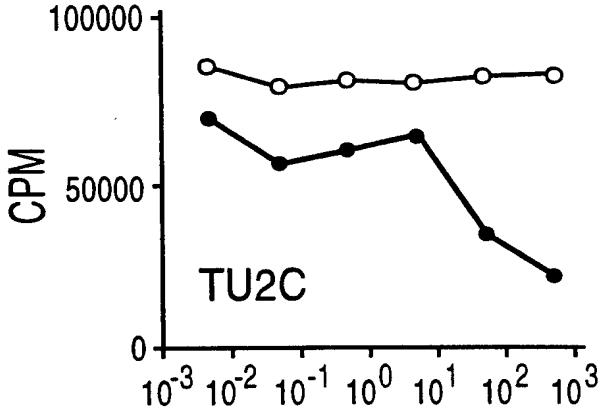

Figure 2C

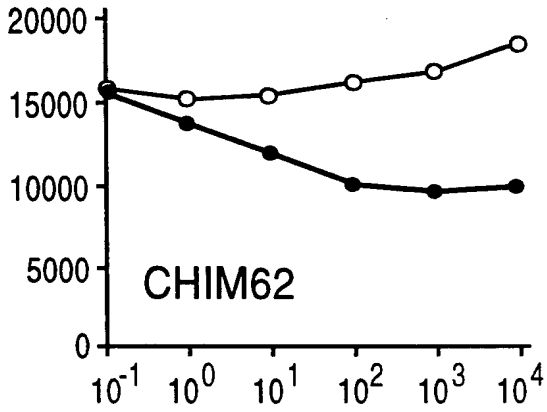

Figure 2D

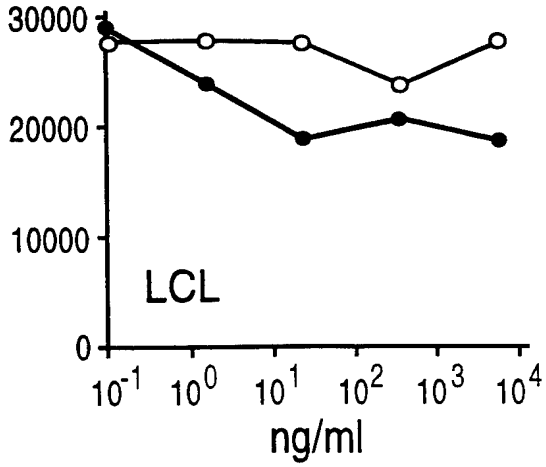

Figure 2E

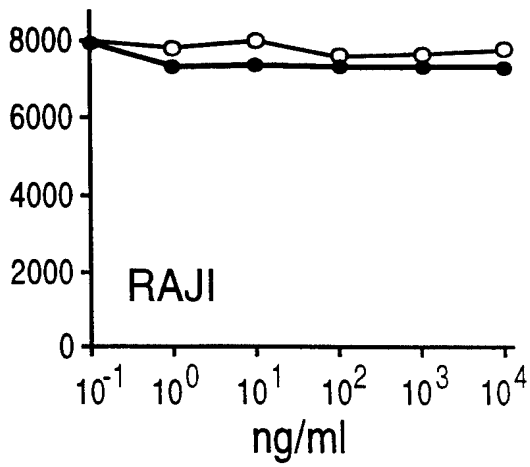

Figure 2F

RECTIFIED SHEET (RULE 91)

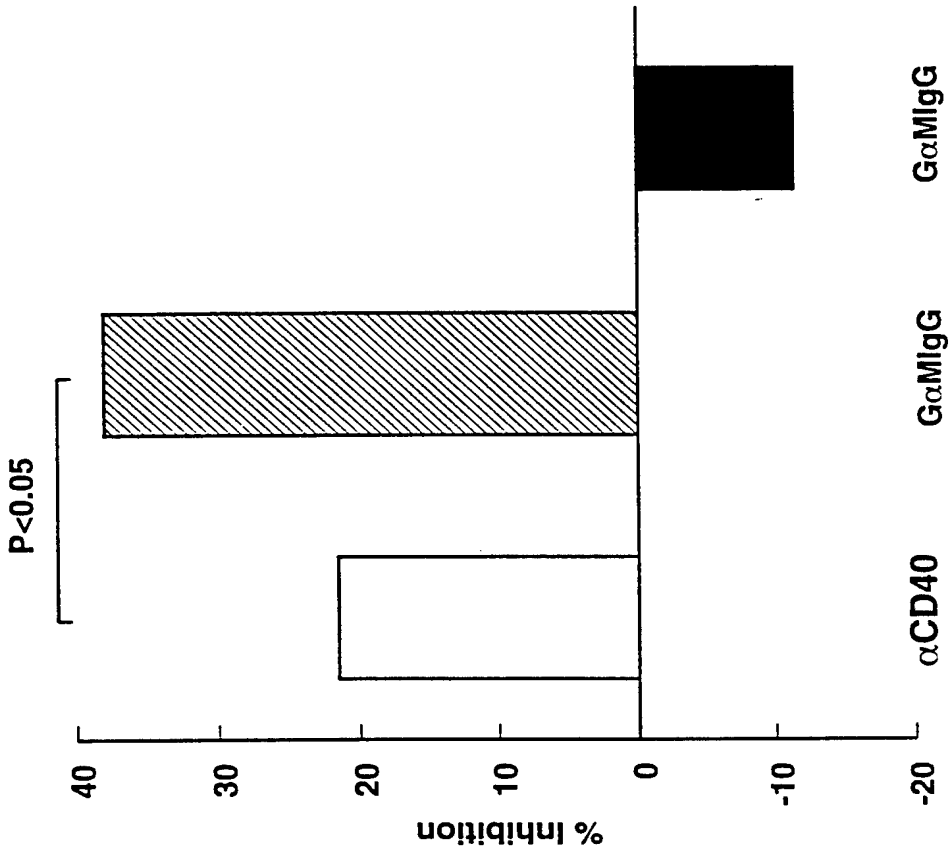

FIGURE 3B

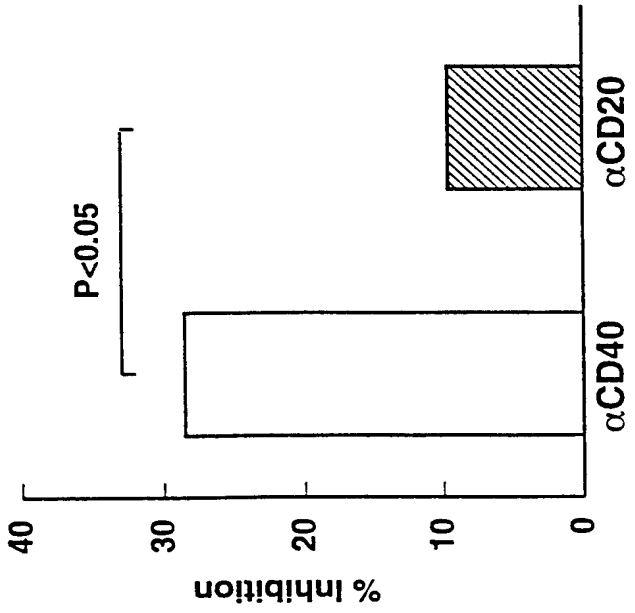

FIGURE 3A

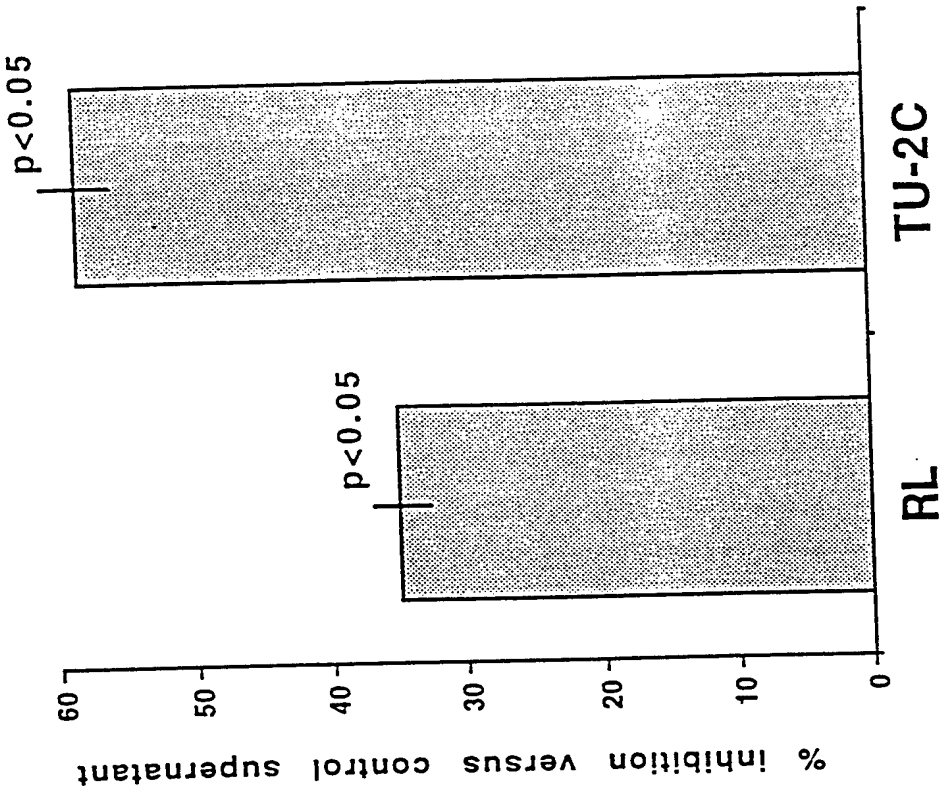

FIGURE 4

RECTIFIED SHEET (RULE 91)

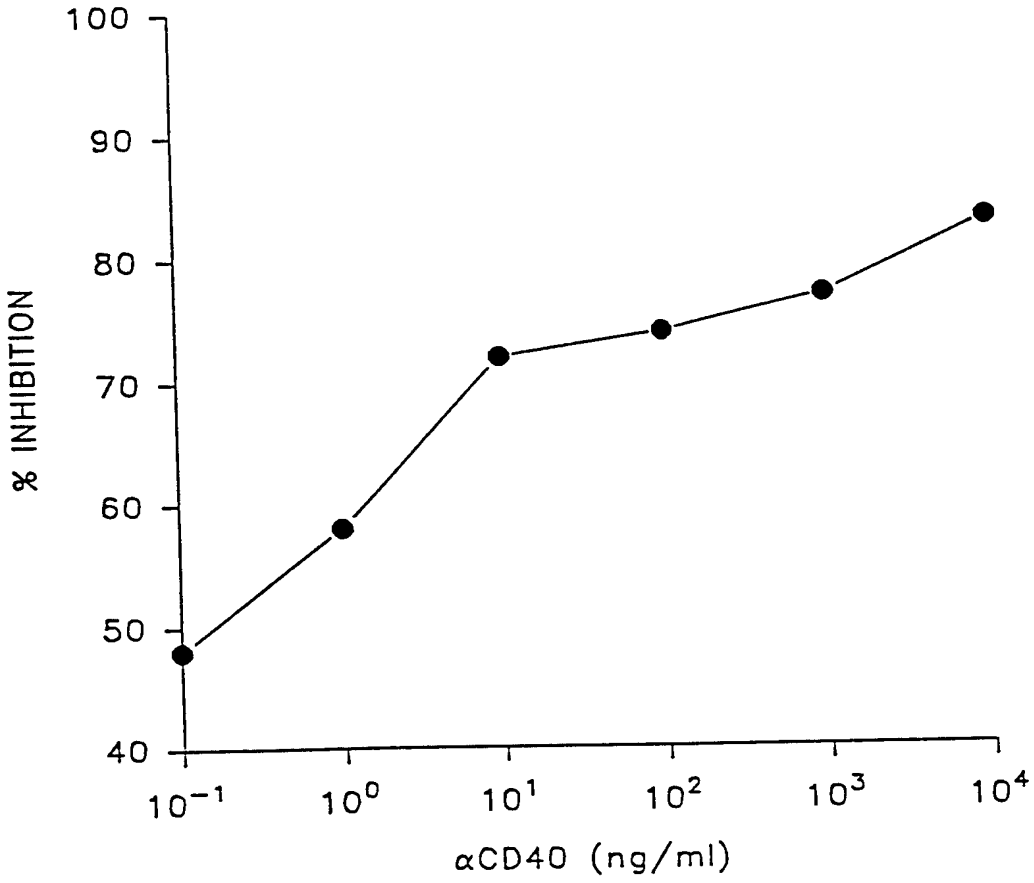
100
90
80
70
60
50
40
% INHIBITION
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
αCD40 (ng/ml)

FIGURE 5

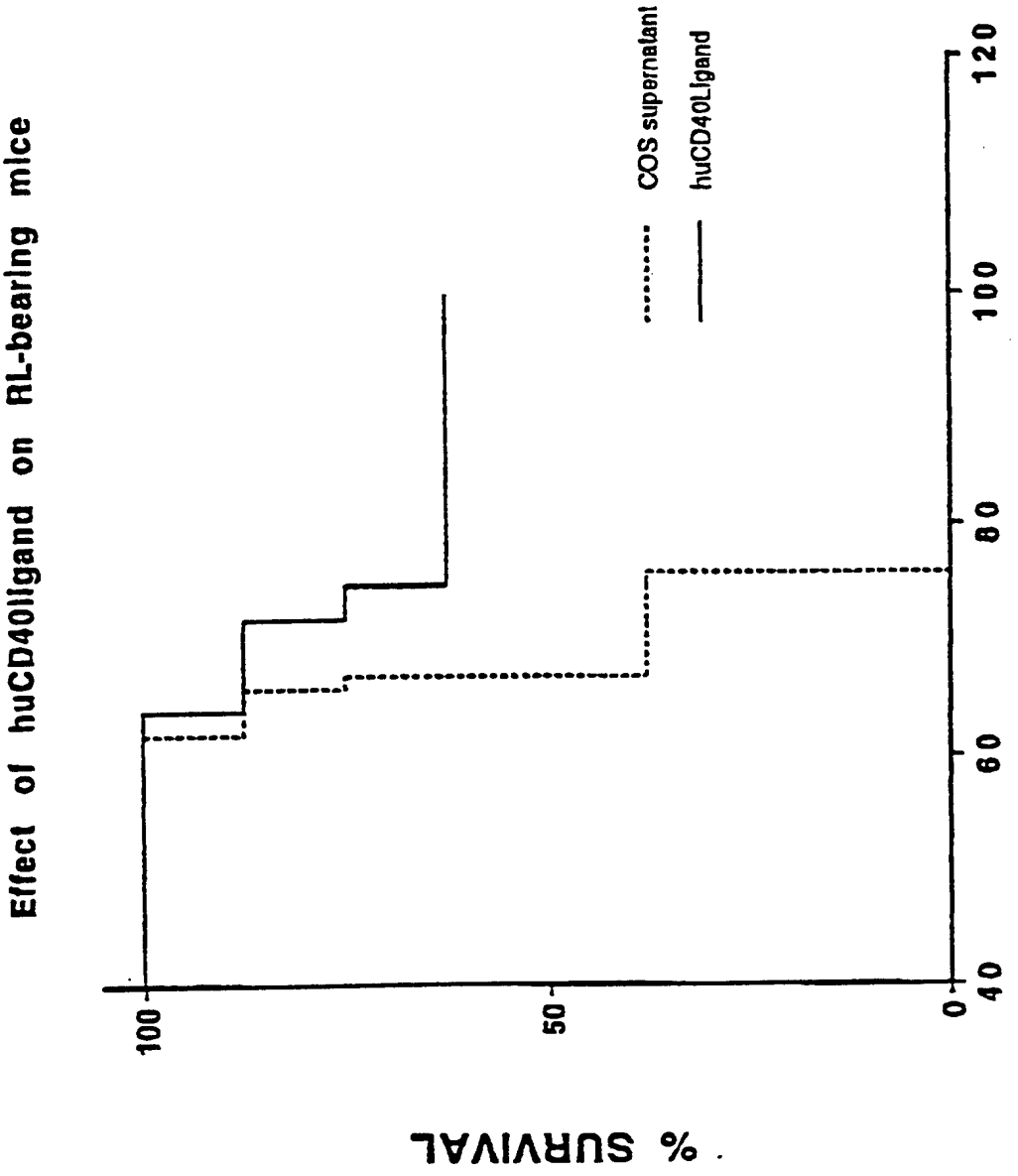
Effect of huCD40ligand on RL-bearing mice
COS supernatant
huCD40Ligand
% SURVIVAL
100
50
0
40
60
80
100
120
DAY OF DEATH

FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14767

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 134.1, 136.1, 138.1, 141.1, 143.1, 144.1,; 435/252.3, 320.1; 514/2, 8; 530/350, 351, 388. 22, 380.73

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, EMBASE, CHEM ABSTRACTS, MEDLINE, WPI
SEARCH TERMS: CD40, C40 LIGAND, CANCER, TUMOR, NEOPLASIA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,247,069 (LEDBETTER ET AL.) 21 SEPTEMBER 1993, SEE ENTIRE DOCUMENT.	1-31
Y	US, A, 5,182,368 (LEDBETTER ET AL.) 26 JUNE 1993, SEE ENTIRE DOCUMENT.	1-31
Y	PERIOD BIOL. VOLUME 92, NUMBER 1, ISSUED 1990, JOVANOVIC ET AL. "THE ROLE OF CD40 IN THE PROLIFERATION OF CLL B LYMPHOCYTES", PAGES 89-90, SEE ENTIRE DOCUMENT.	1-31
Y	KNAPP ET AL., "LEUKOCYTE TYPING IV", PUBLISHED 1989 BY OXFORD UNIVERSITY PRESS, PAGES 90-91, SEE ENTIRE DOCUMENT.	1-31

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
19 FEBRUARY 1995

Date of mailing of the international search report
03 APR 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14767

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEUKEMIA, VOLUME 4, ISSUED 1990, LAW ET AL., "ANALYSIS OF EXPRESSION AND FUNCTION OF CD40 ON NORMAL AND LEUKEMIC HUMAN B CELL PRECURSORS", PAGES 732-738 , SEE ENTIRE DOCUMENT.	1-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/14767

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 39/00, 39/395; C07K 14/00, 14/435, 14/705, 16/00, 16/18, 16/46

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 133.1, 134.1, 136.1, 138.1, 141.1, 143.1, 144.1,; 435/252.3, 320.1; 514/2, 8; 530/350, 351, 388. 22, 380.73